1 **JAST (Journal of Animal Science and Technology) TITLE PAGE**
2 **Upload this completed form to website with submission**
3

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Identification of genomic regions and genes associated with subclinical ketosis in periparturient dairy cows
Running Title (within 10 words)	Genes associated with subclinical Ketosis in Holstein cows
Author	Jihwan Lee[1], KwangHyeon Cho[2], Kent A. Weigel[3], Heather M. White[3], ChangHee Do[4], Inchul Choi[4]
Affiliation	1 Dairy Science Division, National Institute of Animal Science, RDA, Cheon-an, 31000, Korea 2 Department of Beef and Dairy Science, Korea National College of Agriculture and Fisheries, Jeonju-si, Jeollabuk-do, 54874, Korea 3 Department of Animal and Dairy Sciences, University of Wisconsin, Madison, 53706, USA 4 Department of Animal and Dairy Sciences, Chungnam National University, 34134, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Jihwan Lee (http://orcid.org/0000-0002-0040-3104) KwangHyeon Cho (http://orcid.org/0000-0003-1564-5656) Kent A. Weigel (http://orcid.org/0000-0002-2391-6260) Heather M. White (http://orcid.org/0000-0001-5449-2811) ChangHee Do (http://orcid.org/0000-0003-0573-5519) Inchul Choi (https://orcid.org/0000-0001-5011-2658)
Competing interests	The authors declare that they have no conflict of interest
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	Not applicable.
Acknowledgements	This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1A2C1004715 to IC) and the USDA Agriculture and Food Research Initiative Critical Agricultural Research and Extension (CARE; 2015-67028-23572). We also thank to Ryan S. Pralle who helped to collect, combine, and provide the dataset.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Jihwan Lee, KwangHyeon Cho, Inchul Choi Data Curation: KwangHyeon Cho, Inchul Choi Formal Analysis: Jihwan Lee, KwangHyeon Cho, Inchul Choi Methodology: KwangHyeon Cho, Kent A. Weigel, Heather M. White, Inchul Choi Software: KwangHyeon Cho, Inchul Choi Validation: Kent A. Weigel, Heather M. White, Inchul Choi Investigation: ChangHee Do, Inchul Choi Writing - original draft: Jihwan Lee, KwangHyeon Cho, Inchul Choi Writing – review & editing: Jihwan Lee, KwangHyeon Cho, Kent A. Weigel, Heather M. White, ChangHee Do, Inchul Choi
Ethics approval and consent to participate	All experimental protocols were approved by the Animal Care and Use Committee of the College of Agiculture and Life Sciences at the University of Wisconsin-Madison (ACUC no.A005802).

4
5 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Inchul Choi

ACCEPTED

Email address – this is where your proofs will be sent	icchoi@cnu.ac.kr
Secondary Email address	
Address	
Cell phone number	
Office phone number	+82-42-821-5788
Fax number	+82-42-823-2766

6
7

ACCEPTED

Abstract

Subclinical ketosis (SCK) is a prevalent metabolic disorder that occurs during the transition to lactation period. It is defined as a high blood concentration of ketone bodies (beta-hydroxybutyric acid f≥ 1.2 mmol/L) within the first few weeks of lactation, and often presents without clinical signs. SCK is mainly caused by negative energy balance (NEB). The objective of this study is to identify single nucleotide polymorphisms (SNPs) associated with SCK using genome-wide association studies (GWAS), and to predict the biological functions of proximal genes using gene-set enrichment analysis (GSEA). Blood samples were collected from 112 Holstein cows between 5 and 18 days postpartum to determine the incidence of SCK. Genomic DNA extracted from both SCK and healthy cows was examined using the Illumina Bovine SNP50K BeadChip for genotyping. GWAS revealed 194 putative SNPs and 163 genes associated with those SNPs. Additionally, GSEA showed that the genes retrieved by DAVID (Database for Annotation, Visualization, and Integrated Discovery) belonged to calcium signaling, starch and sucrose, immune network, and metabolic pathways. Furthermore, the proximal genes were found to be related to germ cell and early embryo development. In summary, this study proposes several feasible SNPs and genes associated with SCK through GWAS and GSEA. These candidates can be utilized in selective breeding programs to reduce the genetic risk for SCK and subfertility in high-performance dairy cows.

Keywords: Subclinical ketosis, GWAS, SNP, gene-set enrichment analysis, biomarker

ACCEPTED

Introduction

Over the past three decades, intensive genetic selection for high milk yield in dairy cattle has led to a pronounced energy deficit postpartum and a decline in fertility [1, 2]. This occurs because negative energy balance (NEB) takes place when nutrient requirements for maintenance and lactation exceed dietary intake [3]. Cows experiencing NEB in late gestational and early lactation are particularly at risk of metabolic disorders due to utilization of body fat as a source of energy, leading to an elevation of circulating ketone bodies concentration in the bloodstream. Subclinical ketosis (SCK) is a prevalent metabolic disorder in high-producing dairy cows, characterized by increased concentrations of ketone bodies in the b1ood without clinical signs of ketosis [4]. The gold standard diagnostic test for SCK is the measurement of a beta-hydroxybutyric acid (BHBA) in serum/plasma [5, 6] and cows with a BHBA concentration of 1.2 to 2.9 mmol/L are considered to have SCK [6]. The average incidence of SCK within the first weeks of lactation ranges from 26 to 56% [7, 8]. Additionally, Cows with higher milk production were at increased risk for hyperketonemia (HYK), while cows with a body condition score (BCS) of 4 or higher before calving or those that lost more body condition during the transition period were more likely to develop HYK, emphasizing the importance of avoiding over-conditioning of cows during the dry period and excessive BCS loss during the transition period [9].

Interestingly, SCK has a negative association on reproductive performance, including an increased calving to first estrus, first insemination, and pregnancy intervals [2, 10]. Specifically, SCK cows are less likely to become pregnant after first insemination and produced less milk [11-13].

Loor and colleagues reported that 2,415 genes were altered in liver from periparturient dairy cows undergoing nutrition-induced ketosis, and the genes were associated with oxidative phosphorylation, protein ubiquitination, cytokine signaling, fatty acid uptake/transport, fatty acid oxidation, cholesterol metabolism, growth hormone signaling, proton transport, and fatty acid

ACCEPTED

57 desaturation [14]. Additionally, the proteome analysis in feed-deprived dairy cows revealed that
58 altered proteins are involved in fatty acid oxidation, glycolysis, electron transfer, protein degradation,
59 antigen processing, cytoskeletal rearrangement, and cholesterol transport [15]. However, our
60 understanding of polymorphisms within genes linked to SCK, which could aid in the removal of cows
61 with SCK-susceptible genotypes from the breeding population, remains limited. This is due to the low
62 heritability range (0.01~0.16) of the trait and the challenges associated with detecting the phenotypic
63 trait. Nonetheless, it's noteworthy that a reference population, utilizing blood BHB concentration
64 measurements, has the potential to estimate hyperketonemia breeding values for a genomic selection
65 program [16, 17].
66
67 In this study, we conducted a SNP (single nucleotide polymorphism)-based gene-set enrichment
68 analysis (GSEA) to identify candidate genes associated with SCK. We first compared allele
69 frequencies of each SNP between the SCK and non-SCK control groups using genome-wide
70 association studies (GWAS). Subsequently, we used GSEA to better understand the association of
71 genes with SCK susceptibility by identifying biological process or functional pathways. GSEA is a
72 powerful analytical method for interpreting multiple contributing factors affecting complex traits and
73 diseases that can be regulated by different combinations of mutations among different genes within
74 biological groupings [18-20]. The identification of genetic markers and putative biomarker genes
75 using GWAS-GSEA would contribute to successful breeding programs of dairy cattle and provide
76 early and accurate diagnosis in terms of SCK susceptibility.
77

78 Materials and methods

79 All experimental protocols were approved by the Animal Care and Use Committee of the College of
80 Agriculture and Life Sciences at the University of Wisconsin-Madison (ACUC no. A005802).

81 Animals and phenotypic data

82 As previously described in other studies [9, 17], Holstein cows were fed a corn silage and wheat straw
83 based TMR during the dry period, and a TMR based on corn silage and alfalfa silage after calving.
84 Blood samples were collected from a total of 112 Holstein cows two days per week between 5 and 18

ACCEPTED

days postpartum, during the morning feeding, to determine the incidence of SCK. Blood BHBA concentration was measured using an electronic hand-held biosensor, the Precision Xtra meter (Abbott Lab., Abbott Park, IL, USA), which is a useful cow-side ketone test with high sensitivity (91to 94%) for detecting SCK in postpartum dairy cow blood samples, compared with laboratory assays [21]. Cows were diagnosed with SCK (hyperketonaemia) if blood BHBA concentration was $\geq$ 1.2 mmol/L. A binary SCK phenotype was assigned each cow. Of the 112 cows, 30 (26.7%) were diagnosed with SCK, and 82 (71.4%) were healthy.

DNA extraction and genotypic data

Genomic DNA was extracted from hair follicles of cows and genotyped using the Illumina BovineSNP50K BeadChip according to the manufacturer's instructions (Illumina, San Diego, CA, USA), which includes 54,609 Bos Taurus autosome SNPs. The Genome Studio Data Analysis software (Illumina) was used for visualizing SNP data and conducting preliminary analysis. Initially, we compared individual SNPs from SCK cows and healthy cows, and examined alternative alleles or variants at a given SNPs. SNP filtering was performed using PLINK 1.9 software with the following exclusion criteria: minor allele frequency (MAF) <0.01; call rate <0.10; and Hardy–Weinberg equilibrium (HWE) <0.0001. After a quality control process, 43,552 SNPs were retained for further analyses. To identify genomic regions linked with SCK, we utilized a binary trait, assigning '1' when blood BHBA concentration $\geq$ 1.2, and '0' otherwise. The Genetic Relationship Matrix (GRM) that we constructed was then employed in a Genome-Wide Association Study (GWAS) using a following mixed linear model (MLM):

$$y_{ij} = \mu + \beta SNP_i + a_j + e_{ij}$$

where y_{ij} is the binary SCK phenotype for a given cow with overall mean μ; β is the marker genotype effect, SNP_i is the additive SNP substitution effect, a_j is a random polygenic effect, and eij is a vector of random residual effects. Genome-wide p-values threshold of 10^{-5} was used for analyses of variants.

Gene-set enrichment analysis and visualization of gene network

ACCEPTED

111 To assign SNPs to genes, we searched for SNPs that were detected within the genomic sequences of
112 annotated genes (using dbSNP; https://www.ncbi.nlm.nih.gov/SNP/) or within 20 kb of the 5' or 3'
113 ends of the first or last UTR, respectively. To explore functional enrichment in the gene sets and
114 generate KEGG biological pathways, we used DAVID (Database for Annotation, Visualization, and
115 Integrated Discovery) Bioinformatics Resources 6.8 (http://david.abcc.ncifcrf.gov/). To confirm the
116 predicted gene set function and interaction, we analyzed the listed genes in the KEGG pathways using
117 the web-based tool Gene Multiple Association Network Integration Algorithm (GeneMANIA;
118 https://genemania.org/). We targeted SNPs with a call rate of ≥95% and a minor allele frequency
119 (MAF) of ≥5% for prediction of gene sets in this study.

120

121 **Statistical analysis**

122 Minor allele frequency (MAF) was determined using the FREQ procedure of SAS version 9.2 (SAS
123 Institute, Cary, NC, USA). The Benjamini-Hochberg (BH) false discovery rate (FDR) correction was
124 applied to raw p-values (<0.05). The distribution of genotypes was tested for deviation from Hardy-
125 Weinberg equilibrium using a chi-square test. A P-value less than 0.05 was considered statistically
126 significant.

127

128 Results and discussion

129 We conducted GWAS by analysing 54,609 SNPs and identified 8,360 loci that showed differences
130 when comparing SNPs between the SCK and healthy control groups. We then eliminated SNPs with
131 lower MAF frequencies, which were not considered to be genome-wide significant due to the sample
132 size, and identified 194 SNPs associated with SCK in 163 functional candidate genes. This was
133 achieved by evaluating all SNPs within each gene and the surrounding 1 Mb on each side of the gene
134 for their suitability to serve as the gene's proxy (Table 1 and supplemental data 1) [22].

135

136 To identify key genes and pathways associated with SCK, we performed KEGG pathway enrichment
137 analysis using DAVID (https://david.ncifcrf.gov/). We found several pathways that may be involved

ACCEPTED

in the incidence of SCK, including calcium signaling, starch and sucrose metabolism, cAMP signaling, intestinal immune network for IgA production, and metabolic pathways (Table 2), although none of the evaluated KEGG pathways or GO gene sets were significantly enriched for genes associated with diseases such as ketosis. Among these pathways, calcium signaling, starch and sucrose metabolism, and cAMP signaling pathways were statistically significant ($P<0.05$). In the calcium signaling pathway, we identified several candidate genes including ATP2B1 (ATPase Plasma Membrane Ca2+ Transporting 1), STIM2 (Stromal Interaction Molecule 2), PLCD3 (Phospholipase C Delta 3), RYR2 (Ryanodine receptor 2), and PTGFR (Prostaglandin F Receptor). In the starch and sucrose metabolism pathway, three candidate genes were detected: UGT1A6 (UDP Glucuronosyltransferase Family 1 Member A6), UGT2A1 (UDP Glucuronosyltransferase Family 2 Member A1 complex locus), and LOC1002966901 (probable maltase-glucoamylase 2). The cAMP signaling pathway included ATP2B1, TIAM1 (T-cell lymphoma invasion and metastasis 1), RYR2, PDE3A (phosphodiesterase 3A), and MAPK10 (mitogen-activated protein kinase 10). We then analyzed the genes listed in the KEGG pathways (Table 2) using GeneMANIA, which can extend the listed genes with functionally similar genes and predict their biological function/network. This approach showed that the listed genes are co-expressed (78.47%) and co-localized (13.73%). Interestingly, UGT1A6 and UGT2A1 may affect metabolism, such as glucuronidation.

Identification of genes affecting susceptibility to common disease is very difficult because each causal gene only makes a limited contribution to the diseases. Although ketosis is an important metabolic disease of dairy cows, and SCK occurs more often than clinical ketosis, little is known about the genes and signal pathways affecting SCK in the cattle. It has been reported that polymorphisms within the APOBR gene, which codes for the apoliporportein receptor, are associated with the milk level of a prognostic ketosis biomarker in dairy cows [23]. Another study has reported that a SNP in the 3́-untranslated region (UTR) of PRKAG1(Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 1), a regulatory subunit of the AMP-activated protein kinase (AMPK) that plays a critical role in regulating cellular energy metabolism, can influence BHBA levels and milk production. This effects is due to the distortion of the target site of the highly expressed microRNA mir-423-5p in the bovine mammary gland, liver, and kidney [24]. These findings suggest that allele mutation in specific

ACCEPTED

166 genomic regions may be related to the susceptibility of SCK. However, most studies have focused on
167 evaluating the value of phenotypic traits such as the levels of BHBA and NEFA for predicting ketosis
168 [16] rather than identifying specific variants associated with functional candidate genes that influence
169 disease susceptibility.
170 A recent study has reported that a combined approach using GWAS, genome-wide interaction studies
171 (GWIS), and metabolic pathway enrichment analyses identified SNPs and proximal candidate genes
172 associated with susceptibility to hyperketonemia (HYK). This suggest that the combined analysis is an
173 effective way to detect genetic factors contributing to HYK, and that the proposed genes are related to
174 energy and lipoprotein metabolism, particularly insulin secretion or resistance [25].
175
176 In this study, we identified SNPs associated with SCK using GWAS and a pathway-based approach,
177 leading to the discovery of functionally important genes. For example, SNPs closely located to
178 ANAPC4 and SEC 23A are identified by using the combined analysis (GWAS-GSEA). This result is
179 consistent with previous literatures which states that both genes are associated with nutrition-induced
180 ketosis [14] and that expression level of these genes is altered in ketogenic diet rats, suggesting their
181 involvement in mitochondrial biogenesis [26].
182 In addition, several genes listed in Table 1 are involved in metabolic dysfunction such as diabetes;
183 RCAN1 (Regulator of calcineurin 1) is highly expressed in type 2 diabetes in human and mice, and
184 elevation of RCAN1 reduces β-cell mitochondrial function and ATP availability, resulting in a
185 reduction of glucose-stimulated insulin secretion [27], ST8SIA1 (ST8 Alpha-N-Acetyl-Neuraminide
186 Alpha-2,8-Sialyltransferase 1), a membrane-bound glycosphingolipid, is reported to affect impaired
187 glucose-stimulated insulin secretion [28], and an intronic SNP within LINGO2 (Leucine rich repeat
188 and Ig domain containing 2) genes is associated with body mass and adiposity in elder humans [29].
189 Moreover, analysis of integrated function/interaction using GeneMANIA supports our bioinformatics
190 approach. For example, UGT1A6 and UGT2A1 are highly associated with metabolic dysfunction
191 observed in the ketogenic diet in humans and high-fat diet-induced fatty liver in rat [30, 31].
192 In this study, our analysis based on GWAS-GSEA suggested the involvement of calcium signaling,
193 starch and sucrose metabolism, cAMP signaling, intestinal immune network for IgA production, and

ACCEPTED

194 metabolic pathways in SCK. Currently, understanding of the interplay between the metabolic and
195 immune systems is crucial to decipher the integration of metabolism and immunity in periparturient
196 dairy cows. As previously mentioned, the periparturient dairy cow experiences a significant increase
197 in nutrient requirements for lactation; roughly a threefold increase in glucose demand, a twofold
198 increase in amino acids demand, and approximately a fivefold increase in fatty acids [32]. Furthermore,
199 cows require a fourfold increase in calcium on the day of parturition, and early lactating cows
200 experience negative calcium balance [33, 34]. Intracellular calcium, in particular, must be maintained
201 to avoid nerve and muscle dysfunction, as it is involved in many signaling pathways [35].
202 Additionally, NEB during early lactation may worsen periparturient immunosuppression, making
203 dairy cows more vulnerable to infectious diseases [36]. Hyperketonemia, in particular, appears to
204 negatively affect immune function, impairing udder defense mechanisms related to leukocyte function
205 in NEB cows [37]. Although the mechanisms of impairment have not been fully elucidated, the high
206 concentration of ketone bodies has been associated with suppressed bovine lymphocyte blastogenesis,
207 lowered chemotactic capacity of leukocytes, and decreased production of INF-gamma and TNF alpha
208 [38-40].
209 Moreover, we identified several candidate genes that are related to reproductive performance,
210 including early embryo development, implantation, and germ cell development. For instance,
211 expression of EDARADD (Ectodysplasin-A receptor-associated adapter protein) and LINGO2
212 (Leucine rich repeat and Ig domain containing 2) affect early cleaving embryos development and
213 oocyte maturation, respectively [41-43]. SMG6 (SMG6 Nonsense Mediated mRNA Decay Factor)
214 depleted blastocyst embryos did not form the ICM, leading to early embryonic lethality in mice [44].
215 Additionally, ATP2B1 (ATPase plasma membrane Ca2+ transporting 1) and GABPA (GA Binding
216 Protein Transcription Factor Subunit Alpha) may affect spermatogenesis via sperm calcium channel
217 and folliculogenesis via granulosa cell interactions, respectively [45, 46]. Importantly, a SNP located
218 within an intron of MUC19 (Mucin glycoprotein 19) has been reported to be involved in early
219 embryonic loss during the implantation window of heifer Holstein cows [42].
220

ACCEPTED

221 In summary, our study identified chromosomal regions associated with the incidence of SCK and their
222 corresponding genes, highlighting the relationship between nutritional metabolism and immunity in
223 periparturient dairy cows through GSEA. Our approach using SNP and GSEA provided integrated
224 information for the identification of genes associated with SCK. Notably, we also found that the
225 proximal genes of SNPs are related to gametogenesis, early preimplantation development, and the
226 implantation window, suggesting potential links between SCK and reproductive performance. This
227 genetic information can be utilized for optimizing breeding programs and managing high-performance
228 cows with infertility, thus minimizing economic losses caused by SCK.

ACCEPTED

References

1. Lucy MC. Reproductive loss in high-producing dairy cattle: Where will it end? J Dairy Sci. 2001;84(6):1277–93. Available from: http://dx.doi.org/10.3168/jds.S0022-0302(01)70158-0

2. Walsh RB, Walton JS, Kelton DF, LeBlanc SJ, Leslie KE, Duffield TF. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J Dairy Sci. 2007;90(6):2788–96. Available from: http://dx.doi.org/10.3168/jds.2006-560

3. Drackley JK, Cardoso FC. Prepartum and postpartum nutritional management to optimize fertility in high-yielding dairy cows in confined TMR systems. Animal. 2014;8(SUPPL. 1):5–14.

4. Andersson L. Subclinical ketosis in dairy cows. Vet Clin North Am Food Anim Pract. 1988;4(2):233–51. Available from: http://dx.doi.org/10.1016/S0749-0720(15)31046-X

5. Duffield TF, Sandals D, Leslie KE, Lissemore K, McBride BW, Lumsden JH, et al. Efficacy of Monensin for the Prevention of Subclinical Ketosis in Lactating Dairy Cows. J Dairy Sci. 1998;81(11):2866–73. Available from: http://dx.doi.org/10.3168/jds.S0022-0302(98)75846-1

6. Oetzel GR. Monitoring and testing dairy herds for metabolic disease. Vet Clin North Am - Food Anim Pract. 2004;20(3):651–74.

7. Raboisson D, Mounié M, Maigné E. Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: A meta-analysis and review. J Dairy Sci. 2014;97(12):7547–63. Available from: http://dx.doi.org/10.3168/jds.2014-8237

8. Suthar VS, Canelas-Raposo J, Deniz A, Heuwieser W. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J Dairy Sci. 2013;96(5):2925–38. Available from: http://dx.doi.org/10.3168/jds.2012-6035

9. Rathbun FM, Pralle RS, Bertics SJ, Armentano LE, Cho K, Do C, et al. Relationships between body condition score change, prior mid-lactation phenotypic residual feed intake, and hyperketonemia onset in transition dairy cows. J Dairy Sci. 2017;100(5):3685–96.

10. Rutherford AJ, Oikonomou G, Smith RF. The effect of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle. J Dairy Sci. 2016;99(6):4808–15.

11. Asl AN, Nazifi S, Ghasrodashti AR, Olyaee A. Prevalence of subclinical ketosis in dairy cattle in the Southwestern Iran and detection of cutoff point for NEFA and glucose concentrations for diagnosis of subclinical ketosis. Prev Vet Med. 2011;100(1):38–43. Available from: http://dx.doi.org/10.1016/j.prevetmed.2011.02.013

ACCEPTED

12. Duffield TF, Lissemore KD, McBride BW, Leslie KE. Impact of hyperketonemia in early lactation dairy cows on health and production. J Dairy Sci. 2009;92(2):571–80. Available from: http://dx.doi.org/10.3168/jds.2008-1507

13. McArt JAA, Nydam D V., Oetzel GR. Epidemiology of subclinical ketosis in early lactation dairy cattle. J Dairy Sci. 2012;95(9):5056–66. Available from: http://dx.doi.org/10.3168/jds.2012-5443

14. Loor JJ, Everts RE, Bionaz M, Dann HM, Morin DE, Oliveira R, et al. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. Physiol Genomics. 2007;32(1):105–16.

15. Kuhla B, Albrecht D, Kuhla S, Metges CC. Proteome analysis of fatty liver in feed-deprived dairy cows reveals interaction of fuel sensing, calcium, fatty acid, and glycogen metabolism. Physiol Genomics. 2009;37(2):88–98.

16. Pryce JE, Parker Gaddis KL, Koeck A, Bastin C, Abdelsayed M, Gengler N, et al. Invited review: Opportunities for genetic improvement of metabolic diseases. J Dairy Sci. 2016;99(9):6855–73.

17. Weigel KA, Pralle RS, Adams H, Cho K, Do C, White HM. Prediction of whole-genome risk for selection and management of hyperketonemia in Holstein dairy cattle. J Anim Breed Genet. 2017;134(3):275–85.

18. Askland K, Read C, Moore J. Pathways-based analyses of whole-genome association study data in bipolar disorder reveal genes mediating ion channel activity and synaptic neurotransmission. Hum Genet. 2009;125(1):63–79.

19. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci. 2005;102(43):15545–50.

20. Torkamani A, Topol EJ, Schork NJ. Pathway analysis of seven common diseases assessed by genome-wide association. Genomics. 2008;92(5):265–72. Available from: http://dx.doi.org/10.1016/j.ygeno.2008.07.011

21. Iwersen M, Falkenberg U, Voigtsberger R, Forderung D, Heuwieser W. Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. J Dairy Sci. 2009;92(6):2618–24. Available from: http://dx.doi.org/10.3168/jds.2008-1795

22. Lee SH, Lee D, Lee M, Ryoo SH, Seo S, Choi I. Analysis of single nucleotide polymorphisms related to heifer fertility in Hanwoo (Korean cattle). Anim Biotechnol. 2020;33(5):964–9. Available from: https://doi.org/10.1080/10495398.2020.1856124

ACCEPTED

23. Tetens J, Heuer C, Heyer I, Klein MS, Gronwald W, Junge W, et al. Polymorphisms within the APOBR gene are highly associated with milk levels of prognostic ketosis biomarkers in dairy cows. Physiol Genomics. 2015;47(4):129–37.

24. Mahmoudi A, Zargaran A, Amini HR, Assadi A, Vajdi Hokmabad R, Eghbalsaied S. A SNP in the 3'-untranslated region of AMPKγ1 may associate with serum ketone body and milk production of Holstein dairy cows. Gene. 2015;574(1):48–52. Available from: http://dx.doi.org/10.1016/j.gene.2015.07.077

25. Pralle RS, Schultz NE, White HM, Weigel KA. Hyperketonemia gwas and parity-dependent SNP associations in holstein dairy cows intensively sampled for blood β-hydroxybutyrate concentration. Physiol Genomics. 2020;52(8):347–57.

26. Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, et al. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. Ann Neurol. 2006;60(2):223–35.

27. Peiris H, Duffield MD, Fadista J, Jessup CF, Kashmir V, Genders AJ, et al. A Syntenic Cross Species Aneuploidy Genetic Screen Links RCAN1 Expression to β-Cell Mitochondrial Dysfunction in Type 2 Diabetes. PLoS Genet. 2016;12(5):1–24.

28. Bonner-Weir S, Aguayo-Mazzucato C. Physiology: Pancreatic β-cell heterogeneity revisited. Nature. 2016;535:365–6.

29. Rask-Andersen M, Almén MS ällma., Lind L, Schiöth HB. Association of the LINGO2-related SNP rs10968576 with body mass in a cohort of elderly Swedes. Mol Genet Genomics. 2015;290(4):1485–91.

30. Van Der Louw EJTM, Desadien R, Vehmeijer FOL, Van Der Sijs H, Catsman-Berrevoets CE, Neuteboom RF. Concomitant lamotrigine use is associated with decreased efficacy of the ketogenic diet in childhood refractory epilepsy. Seizure. 2015;32:75–7. Available from: http://dx.doi.org/10.1016/j.seizure.2015.09.007

31. Zhang L, Chu X, Wang H, Xie H, Guo C, Cao L, et al. Dysregulations of UDP-glucuronosyltransferases in rats with valproic acid and high fat diet induced fatty liver. Eur J Pharmacol. 2013;721:277–85. Available from: http://dx.doi.org/10.1016/j.ejphar.2013.09.024

32. Bell AW. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J Anim Sci. 1995;73(9):2804–19.

33. Horst RL, Goff JP, Reinhardt TA. Calcium and Vitamin D Metabolism in the Dairy Cow. J Dairy Sci. 1994;77(7):1936–51.

ACCEPTED

34. Horst RL, Goff JP, Reinhardt TA, Buxton DR. Strategies for Preventing Milk Fever in Dairy Cattle. J Dairy Sci. 1997;80(7):1269–80. Available from: http://dx.doi.org/10.3168/jds.S0022-0302(97)76056-9

35. Goff JP, Horst RL. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders. J Dairy Sci. 1997;80(7):1260–8. Available from: http://dx.doi.org/10.3168/jds.S0022-0302(97)76055-7

36. Mallard BA, Dekkers JC, Ireland MJ, Leslie KE, Sharif S, Vankampen CL, et al. Alteration in Immune Responsiveness during the Peripartum Period and Its Ramification on Dairy Cow and Calf Health. J Dairy Sci. 1998;81(2):585–95. Available from: http://dx.doi.org/10.3168/jds.S0022-0302(98)75612-7

37. Suriyasathaporn W, Heuer C, Noordhuizen-Stassen EN, Schukken YH. Hyperketonemia and the impairment of udder defense : a review. Vet Res. 2000;31(4):397–412.

38. Sato S, Suzuki T, Okada K. Suppression of lymphocyte blastogenesis in cows with puerperal metritis and mastitis. J Vet Med Sci. 1995;57(2):373–5.

39. Suriyasathaporn W, Daemen AJJM, Noordhuizen-Stassen EN, Dieleman SJ, Nielen M, Schukken YH. β-Hydroxybutyrate levels in peripheral blood and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes. Vet Immunol Immunopathol. 1999;68:177–86.

40. Zdzisińska B, Filar J, Paduch R, Kaczor J, Lokaj I, Kandefer-Szerszeń M. The influence of ketone bodies and glucose on interferon, tumor necrosis factor production and NO release in bovine aorta endothelial cells. Vet Immunol Immunopathol. 2000;74:237–47.

41. Haines BP, Rigby PWJ. Expression of the Lingo/LERN gene family during mouse embryogenesis. Gene Expr Patterns. 2008;8(2):79–86.

42. Li Y, Liu H, Yu Q, Liu H, Huang T, Zhao S, et al. Growth Hormone Promotes in vitro Maturation of Human Oocytes. Front Endocrinol. 2019;10(7):1–8.

43. Rogers NT, Halet G, Piao Y, Carroll J, Ko MSH, Swann K. The absence of a Ca2+ signal during mouse egg activation can affect parthenogenetic preimplantation development, gene expression patterns, and blastocyst quality. Reproduction. 2006;132(1):45–57.

44. Li T, Shi Y, Wang P, Guachalla LM, Sun B, Joerss T, et al. Smg6/Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay . EMBO J. 2015;34(12):1630–47.

45. MacLean JA, Rao MK, Doyle KMH, Richards JAS, Wilkinson MF. Regulation of the Rhox5 homeobox gene in primary granulosa cells: Preovulatory expression and dependence on SP1/SP3 and GABP. Biol Reprod. 2005;73(6):1126–34.

ACCEPTED

46. Okunade GW, Miller ML, Pyne GJ, Sutliff RL, O'Connor KT, Neumann JC, et al. Targeted ablation of plasma membrane Ca2+-ATPase (PMCA) 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4. J Biol Chem. 2004;279(32):33742–50. Available from: http://dx.doi.org/10.1074/jbc.M404628200

ACCEPTED

Table 1. Top 20 ranked SNPs and functional candidate genes associated with SCK using GWAS.

rs_number	Gene	Chromosome	Position_SNP	p_value
rs41668260	LRRTM4	11	59319163	0.0003
rs43681489	RCAN1	11	59351201	0.0005
rs42034762	HSBP1L1	24	785766	0.0009
rs109448272	ST8SIA1	5	88334676	0.0024
rs41582549	LSAMP	1	61069157	0.0027
rs41603224	TMEM9B	15	44224325	0.0045
rs29027118	AAK1	11	67944693	0.0047
rs109902840	LOC104975886	25	29977087	0.006
rs110679980	EDARADD	28	9131855	0.0062
rs42653943	LINGO2	8	15734273	0.0068
rs42404957	TOX	14	26926569	0.0101
rs109853479	SMG6	19	23798617	0.011
rs109530663	LOC104972363	5	21947260	0.0114
rs42329164	CNTN5	15	9792729	0.0142
rs109007144	ATP2B1	5	19594448	0.0146
rs110298601	SPECC1L	17	73627135	0.0146
rs29022825	GABPA	1	10056851	0.0167
rs110686608	SMG6	19	23761130	0.0172
rs110957256	SPIDR	14	20759946	0.0183
rs42328461	CNTN5	15	9814478	0.0218
rs41656716	MUC19	5	40580237	0.0223

ACCEPTED

Table 2 List of KEGG Pathways using GESA-SNP analysis

Category	Term	P Value	Genes
KEGG_PATHWAY	bta04020: Calcium signaling pathway	0.038	ATP2B1, STIM2, PLCD3, RYR2, PTGFR
KEGG_PATHWAY	bta00500: Starch and sucrose metabolism	0.040	UGT1A6, UGT2A1, LOC100296901
KEGG_PATHWAY	bta04024: cAMP signaling pathway	0.046	ATP2B1, TIAM1, RYR2, PDE3A, MAPK10
KEGG_PATHWAY	bta04672: Intestinal immune network for IgA production	0.055	TNFRSF13B, MAP3K14, CXCL12
KEGG_PATHWAY	bta01100: Metabolic pathways	0.071	UGT1A6, GLUL, SEPHS1, FUT8, CYP27A1, COX7B2, GADL1, ST8SIA1, PLCD3, UGT2A1, AMPD3, EXT2, SARDH, LOC100296901

ACCEPTED